CHIMIA 52 (1998) Nr. 1/2 (Januar/Februar)

Chimia 52 (1998) 56–62
© Neue Schweizerische Chemische Gesellschaft
ISSN 0009–4293

Neurotoxins from Snake Venom

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Abstract. Found within snake venoms are a variety of toxic and nontoxic proteins. The effects of snake venoms depend on all of the components of that venom. However, the lethal effects are usually related to the most potent toxins found within the venom; the two most toxic proteins found in snake venom are neuro- and cardiotoxins. This article reviews a variety of neurotoxins found in snake venom. The range of neurotoxins present in snake venom is varied and includes: peripheral and noncentral, presynaptic, postsynaptic, potassium-channel inhibiting, muscarinic transmission inhibiting, and anticholinesterase types.

Because a snake venom contains many different proteins, some are highly toxic, some are weakly toxic, and some are nontoxic. Toxicity of a snake venom is due to a combinational effect of all components present. However, some snake venoms are more toxic than others because they contain more lethal components. In this review article, the most toxic neurotoxin is discussed.

There are several types of neurotoxins and their structures; the site of action and the mechanism are not identical. Snake neurotoxins are peripheral neurotoxins, rather than centrally neurotoxic; apparently, they do not pass through the blood-brain barrier.

1. Presynaptic Neurotoxins

The presynaptic neurotoxins are also called β-toxins in contrast to postsynaptic or α-toxins. When a β-toxin is added to the neuromuscular preparation, the muscle contraction starts without stimulation of the nerve axon. β-Toxin usually does not affect the depolarization of the muscle itself or have a binding ability to the acetylcholine receptor. It is thus clear that the β-toxin somehow affects the presynaptic end of the nerve and initiates the release of acetylcholine and then eventually stops the release.

At the end of the nerve terminal, there are many vesicles containing acetylcholine. β-Bungarotoxin (β-btx) induces the influx of Ca^{2+} to the nerve terminal; this in turn causes the exocytosis of the nerve transmitter. The exocytosis is made by the membrane fusion of the vesicle and the nerve-terminal membranes. *In vitro*, it was demonstrated that β-btx mediated the fusion of liposome. The result of *in vitro* fusion may explain *in vivo* exocytosis [1].

This can be clearly seen by observing the change in the miniature end-plate potential (MEPP). The MEPP is a very small potential, observed in the neuromuscular junction, that is due to the natural leakage of acetylcholine from the vesicle. When β-toxin is applied, the MEPP frequently decreases first (5–10 min), then suddenly increases (for several hours). Finally, the frequency decreases until it becomes zero.

There are several types of presynaptic toxins. They are usually structurally distinct among themselves. However, there is one common property, and that is the possession of phospholipase A activity. The toxic phospholipase A is usually a basic protein. *Tsai et al.* [2] found that the basic amino acid tended to cluster near the surface region at the NH_2-terminal side in basic phospholipase A.

One type of presynaptic toxin is composed of two subunits bound together. The basic subunit has phospholipase A activity, whereas the acidic subunit has no enzyme activity. Crotoxin is the first presynaptic toxin isolated from snake venom.

The role of the acidic subunit A is to guide the toxin to a specific site; then the basic subunit B functions as a presynaptic toxin [3]. Each subunit alone is relatively nontoxic, but combined, the toxicity is greatly enhanced [4]. The undissociated crotoxin itself shows phospholipase A activity, indicating the active site of subunit B is not masked by subunit A [5]. Besides neurotoxicity, subunit B also has hemolytic activity. Subunit B attaches to many parts of the erythrocyte membranes [6] and also on the postsynaptic membrane [7], in addition to the presynaptic binding site. From a structural viewpoint, both subunits A and B are in the isoforms [8a–c].

Mojave toxin from *Crotalus scutulatus* is also structurally similar to crotoxin and is composed of two subunits [9a, b]. There are considerable amino-acid sequence homologies between the two toxins [10a, b]. Mojave toxin inhibits calcium-channel dihydropyridine receptor binding in rat brain [11].

Crotoxin's neurotoxic action is very similar to β-btx, but has some difference. For instance, crotoxin and its subunit B have a postsynaptic effect, whereas β-btx has no such activity [7].

The acidic and basic subunit-types presynaptic toxins are fairly common in neurotoxic snake venoms. For instance, such toxins have been isolated from the venoms of *C. viridis concolor* [12] and *C. durissus collilineatus* [13]. The amino-acid sequence of the basic subunit also has considerable homology to other snake-venom phospholipases A.

The second type of presynaptic neurotoxin from a chemical viewpoint is that two polypeptide chains are connected by a disulfide bond. The most typical toxin of this type is β-btx. It consists of two chains: The A-chain has 120 amino acids, and the B-chain has 60 amino-acid residues. The amino-acid sequence of the A-chain is similar to the phospholipase A sequence and, in fact, the A-chain does possess phospholipase A activity. For presynaptic activity, phospholipase A activity is essential. For instance, when Ca^{2+} is replaced with Sr^{2+}, phospholipase A activity and presynaptic activity are both lost.

Oxidation of methionine at the 6- and 8-positions lowered the toxicity without affecting antigenicity. Moreover, the NH_2-terminal region of the A-chain plays a crucial role in maintaining functional activity [14]. Heterodimeric β-btx was examined by X-ray diffraction [15].

The exact mechanism of β-btx is not yet known. But it may be that phospholipase A creates a hole in the nerve-end membrane and Ca^{2+} flows to the cytoplasm. As a result, vesicles containing the nerve transmitter acetylcholine discharge it. The nucleotide-sequence-encoding β-btx A_2-chain has been determined [16]. Only the B-subunit with phospholipase A_2 activity had the ability to release acetylcholine and the noncatalytic unit of A-subunit has no action [17]. *Yang* and *Chang* [18] reported that phospholipase A_2 activity and presynaptic toxic activity can be separated.

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CHIMIA 52 (1998) Nr. 1/2 (Januar/Februar)

The third type of presynaptic toxin is a tertiary complex of three polypeptide chains. Taipoxin from the venom of the Australian snake, taipan, has three subunits, α, β, and γ, with an M_r of 46 000. The number of amino-acid residues present in the subunits is 120, 120, and 135, respectively. The α-chain is basic and has phospholipase A activity.

The fourth type is a quaternary complex of four polypeptide chains. Textilotoxin isolated from *Pseudonaja textilis* consists of A, B, C, and D subunits. Subunit D consists of two identical covalently linked polypeptide chains [19a–c].

The last type is a single polypeptide-chain presynaptic neurotoxin. An example of this is notexin from the venom of *Notechis scutulatus scutulatus*, consisting of 119 amino-acid residues with seven disulfide bonds. It has an M_r of 13400. Notexin has isotoxins; they differ in only one amino-acid residue among the two isotoxins [20]. The three-dimensional structure of notexin was determined by crystallography. The core of the protein is very similar to other phospholipases A. The difference, however, exists mainly in the area of residues 56–80 and 85–89 [21].

Not every presynaptic toxin is identical in relation to the release of acetylcholine from the presynaptic site. With β-btx, there is an initial burst of acetylcholine, but eventually the release is stopped. Even though toxins may behave like β-btx, the length of time for acetylcholine release is different for each toxin. Some presynaptic toxins do not release acetylcholine from the beginning and simply stop the release. In such an event, the depolarization wave never reaches the muscle, and the muscle is paralyzed.

A. halys pallas venom contains three types of phospholipase A; they are acidic, neutral, and the basic. The neutral phospholipase A_2 has presynaptic toxic action and it was designated as agkistrodon toxin [22]. Coral snake (*Micrurus nigrocinctus nigrocinctus*) venom contains both pre- and postsynaptic toxins, and the presynaptic toxin is phospholipase A_2 [23]. Removal of the N-terminal octapeptide of the phospholipase A_2 subunit caused the disintegration of the low-affinity Ca^{2+} binding site. The neurotoxicity and the enzymatic activity of β-btx were lost [24].

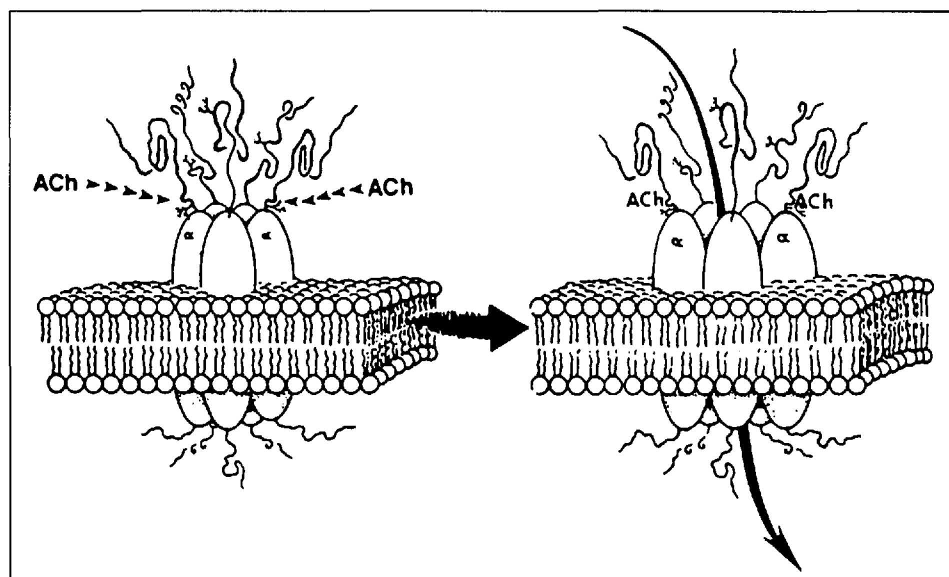


Fig. 1. *The role of AChR in muscle depolarization*

2. Postsynaptic Toxins

2.1. *Nicotinic Type*

Postsynaptic neurotoxins are commonly found in the venoms of Hydrophiidae and Elapidae. The toxins affect the neuromuscular junction at the postsynaptic site by combining with acetylcholine receptor (AChR). These neurotoxins act on the muscle side, rather than the nerve side. The so-labeled *postsynaptic neurotoxins* are really the toxins affecting the particular site of the muscle and should not have been designated neurotoxins. Actually, postsynaptic neurotoxins bind to the acetylcholine receptor in the muscle that is to receive the neurotransmitter acetylcholine. On the other hand, the attachment of acetylcholine to the acetylcholine receptor is considered a part of the nerve-transmitter mechanism. From this functional viewpoint, it is not unreasonable to call them postsynaptic neurotoxins because of their activity. Therefore, the paralysis of the muscle by postsynaptic neurotoxin poisoning is essentially due to the formation of an acetylcholine-receptor neurotoxin complex. One should realize that usually a snake venom contains multiple numbers of neurotoxins. *Bungarus multicinctus* venom is well known as the source of α- and β-btx, but the venom contains many other neurotoxins. For instance, toxin F, which also blocks neuronal nicotinic receptors, has been isolated [25]. Venom from a similar snake, *B. fasciatus*, also contains various neurotoxins [26a, b]. Sea snake, *Acalytophis peronii*, venom also contains major and minor neurotoxins. The only difference between the major and minor postsynaptic toxins is in the 43rd residue. The major toxin at this position contains glutamine, whereas the minor toxin contains glutamic acid [27a, b].

When a normal nerve impulse (depolarization wave) passes through the axon and reaches the end of that axon, the calcium-ion concentration is increased and the neurotransmitter, acetylcholine (ACh), is suddenly released from the vesicle at the end of the nerve. Acetylcholine moves across the synaptic crevice and reaches the acetylcholine receptor in the muscle. The AChR is composed of five subunits, α, α, β, γ, δ. When two molecules of acetylcholine attach to the α-subunits, the AChR changes configuration and becomes an open ion channel, permitting certain ions to pass through (*Fig. 1*). By this mechanism, the depolarization wave reaches the muscle.

The structure of postsynaptic neurotoxins is well studied. There are actually two types of these neurotoxins (*Fig. 2, A*, and *C*). One type has four disulfide bonds (called type I or short-chain neurotoxins). The short-chain neurotoxin has one or two amino acids in segment 8, whereas the long-chain neurotoxins have a longer segment 8 (*Fig. 2*). Another difference is that there is only one amino acid within segment 5 of the short-chain neurotoxin, whereas the long-chain neurotoxin has three amino-acid residues within the segment (*Fig. 2*).

Both short- and long-chain neurotoxins have the same biological activity; namely, to bind to AChR, but there is some difference in chemical properties. It was well documented that the invariant tryptophan residue in short-chain neurotoxin is essential, because the chemical modification of this residue caused the loss of neurotoxicity. However, the modification of a tryptophan residue in α-btx, which is a long-chain neurotoxin, did not appreciably change the toxicity [28].

Most neurotoxins isolated from Australian Elapidae venoms were reported as presynaptic neurotoxins, but a postsynaptic one was isolated from *Acanthophis antarcticus* (Australian death adder) [29].

CHIMIA 52 (1998) Nr. 1/2 (Januar/Februar)

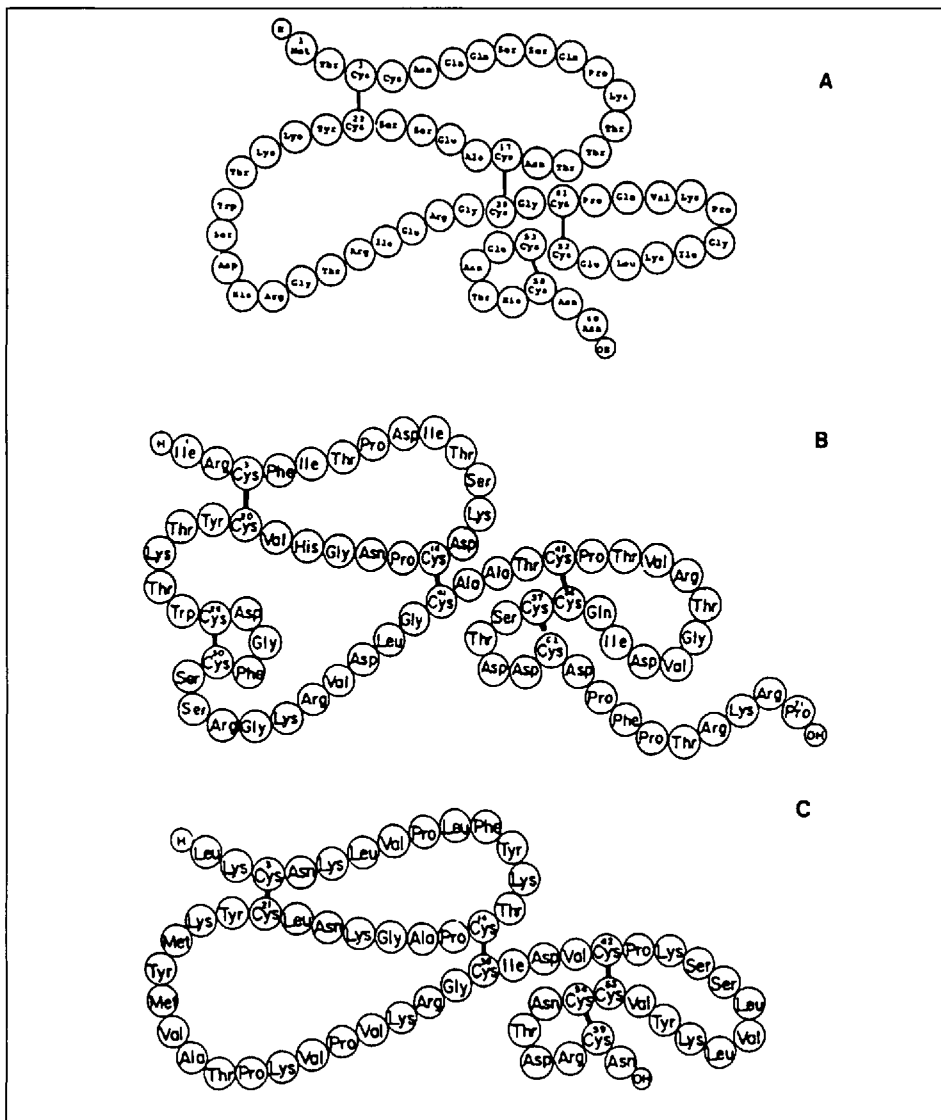


Fig. 2. *Examples of neurotoxins (A, C) and a cardiotoxin (B). A)* Primary structure of lapemis toxin short-chain postsynaptic neurotoxin. *B)* Cardiotoxin from *Naja naja* venom. *C)* Toxin B from *Naja* venom.

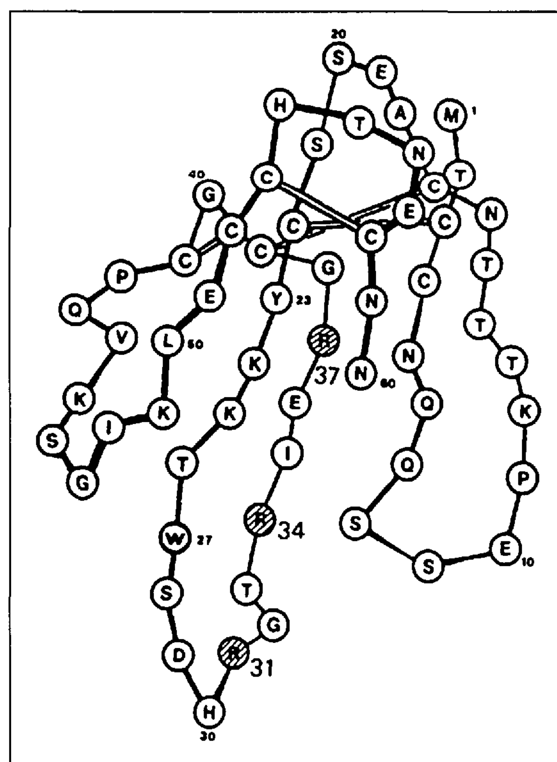


Fig. 3. *Chemical structure of lapemis toxin showing three main loops A, B, and C*

One interesting aspect from a structural viewpoint is that the two types of postsynaptic neurotoxins are very similar to Elapidae venom cardiotoxins (see *Fig. 2, B*). Cardiotoxins stop the heartbeat when they make contact with the heart. Cardiotoxins have four disulfide bonds and a very short segment 8. In this manner, they are similar to short-chain neurotoxins. Although the similarity in disulfide bonds and the peptide backbone is remarkable for cardiotoxins and postsynaptic neurotoxins, there are considerable differences between them in amino-acid composition and sequences. Cardiotoxins do not bind to the AChR, whereas there is strong binding between the neurotoxins and the AChR. The hydrophilic index of cardiotoxins shows them to be quite hydrophobic molecules, whereas the neurotoxins are quite hydrophilic molecules. Cardiotoxins are more general toxins, affecting cell membranes, whereas neurotoxins are specific toxins, binding to acetylcholine receptors.

Postsynaptic neurotoxins are composed mainly of an antiparallel β-sheet and a β-turn structure, with only a small amount of α-helical structure [30a–f]. The toxin is comprised of three loops, A, B, and C (*Fig. 3*). The central loop is considered most important, and it is believed that this loop is attached to the acetylcholine-binding site of the AChR.

The amino-acid sequences of over 100 postsynaptic neurotoxins have been determined by many investigators; therefore, the sequence of all the toxins will not be discussed. However, one should be aware of the incorrect sequence of α-btx, as originally reported earlier [31]. The correct primary structure of α-bungarotoxin was later established [32]. The original paper [31] reported the sequences of Ile-Pro-Ser (9–11), His-Pro (67–68), and Arg-Gln (71–72). However, these sequences are incorrect, and the correct sequences have now been established as Ser-Pro-Ile (9–11), Pro-His (67–68), and Gln-Arg (71–72) by *Ohta et al.* [32].

Snake venoms also contain nonneurotoxic proteins with structures very similar to a postsynaptic neurotoxin. For instance, mambia is a platelet aggregation inhibitor isolated from the venom of *Dendroaspis jamesonii*. It has 59 amino-acid residues with four disulfide bonds and a high homology to postsynaptic neurotoxins [33].

Although postsynaptic neurotoxins are small polypeptides with an M_r of *ca.* 6 800, they are antigenic. However, by conjugating neurotoxin to a protein with a higher M_r, antigeneity can be further enhanced [34].

Some antibodies to cobrotoxin bound to the toxin portion insensitive to confor-

CHIMIA 52 (1998) Nr. 1/2 (Januar/Februar)

mational change and some antibodies bound to the conformation-sensitive portion of neurotoxin [35]. Neurotoxins from several Elapidae and Hydrophidae venoms can be differentiated from the binding ability to monoclonal antibodies against *Naja naja oxiana* [36].

The AChR is a pentamer that is comprised of five subunits (two α, one each of β, γ, and δ), and two of them are identical (*Fig. 2*). The receptor is a ligand (acetylcholine)-gated channel protein, allowing ions to pass through when activated. The ligand, acetylcholine, attaches to the α-subunits. Since there are two α-subunits, the stoichiometry of ligand-receptor interaction is 2 mol of acetylcholine per receptor. Postsynaptic toxins attach to the same sites as acetylcholine; however, the AChR receptor fails to form a channel (*Fig. 4*).

Each subunit is a glycoprotein; however, it is not yet clear just what role the polysaccharide, which is present in each subunit, plays. There are several types of polysaccharides in each subunit. One of them is shown here:

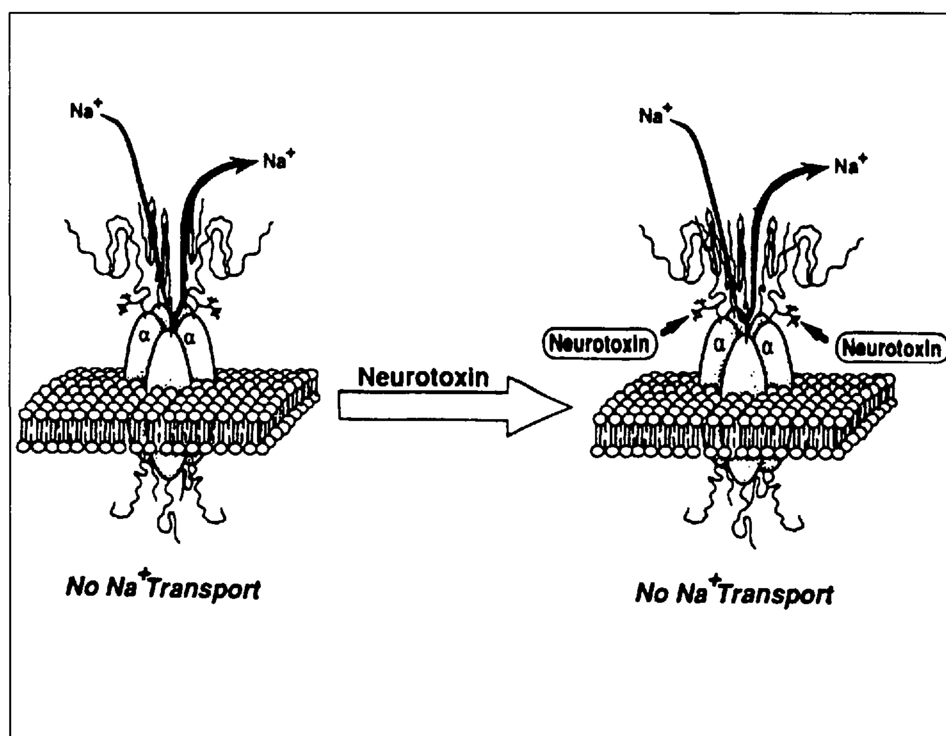
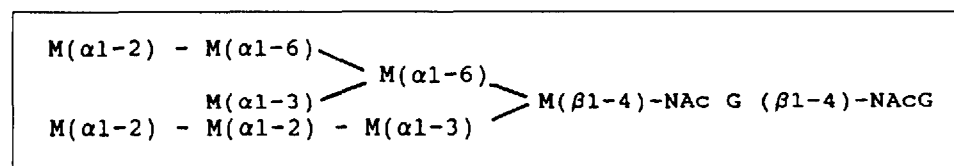


Fig. 4. *Attachment of a neurotoxin to the same site as that of acetylcholine causes the AChR to fail in forming an ion channel in the membrane*



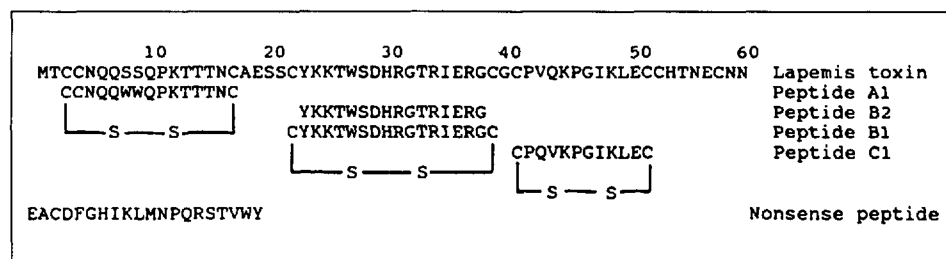
where M is mannose and NAcG is *N*-acetylglucosamine [37].

Neurotoxins actually recognize two fragments of α-subunit of AChR, 128–142 and 185–199 portions [38]. However, a different region of AChR α-subunit, residues 173–204, was reported by *Lentz* [39]. Although it is well recognized that α-subunit is the place for toxin binding, it was reported that β-subunit is also an important determinant in receptor localization and sensitivity to neuronal bungarotoxin [40]. The binding of fragment 185–196 to α-btx was studied by NMR, indicating that this portion is indeed the cholinergic ligand binding site [41]. A simple, nonradioactive, but sensitive, method was developed by *Nomoto et al.* [37], who used horseradish-peroxidase (HRP)-conjugated neurotoxin.

The acetylcholine receptor and a neurotoxin form a noncovalent bond-type complex. The most important question is what portion of a neurotoxin is really involved in the receptor binding. Is it a particular residue, or are several residues involved? *Fig. 3* represents a two-dimensional structure of a postsynaptic neurotoxin – lapemis toxin from *Lapemis hardwickii* – that is based on the X-ray diffraction study of another similar toxin.

From studies of chemical modification of amino-acid residues studied by many investigators, it was shown that the ones located in the central loop are essential for neurotoxicity. For instance, the Arg-31, Arg-34, Trp-27, Tyr-23, Lys-24, and Lys-25 are known to be related to neurotoxicity. It is logical to assume that loop B is most likely to bind to the AChR.

To clarify this problem, synthetic peptides identical with A, B, and C loops were made, and their ability to bind to the acetylcholine receptor was studied [42]. Peptide synthesis:



Only the peptide identical with the central loop B bound to the acetylcholine receptor, whereas the other peptides had no detectable binding. The sulfide bond is essential for binding. When the central loop peptide was reduced and alkylated, the binding ability was lost. This finding suggested that the central loop plays a dominant role in the toxin's ability to bind the receptor.

Although the central loop is the most important one, the other two loops are also essential in order to express full neurotoxic activity. *Lin et al.* [43] showed that Arg-34, 37 at the central loop are essential for neurotoxic activity. The modifications of Lys 50, 56 and Arg 707, 72, Lys-71 at the C-terminal loop and Lys-10 on the N-terminal loop cause the loss of neurotoxicity. These results suggest that synergistic effect of three loops takes place in neurotoxic action.

However, *Lin* and *Chang* [44] reported that modification of arginine residues caused the decrease of lethality, but there was no effect of Arg of α-btx.

Most AChR studies were done using skeletal muscle or torpedo tissues. The acetylcholine-receptor concentration in the brain is very small, but it is present. Recently, the AChR in the brain has been actively studied using snake postsynaptic neurotoxins. Some of these are rather typ-

CHIMIA 52 (1998) Nr. 1/2 (Januar/Februar)

ical neurotoxins that bind to both skeletal muscles and the brain, and some of them are specific to the brain AChR [45]. Since a brain α-subunit of AChR binds to α-btx, there must be a similarity between the toxin-binding site for the brain AChR and the muscle AChR [46a, b].

Neuronal membrane components that bind α-btx with high affinity can increase intracellular levels of free calcium, demonstrating the components' function as nicotinic receptor [47]. Even the retina contains the AChR that is sensitive to α-btx [48].

2.2. *Muscarinic Type*

There are two types of acetylcholine receptors, those responsive to muscarine (mAChR) and those responsive to nicotine (nAChR). The mAChR is present in the brain, various smooth muscles, cardiac muscle, and exocrine glands; it is classified into five subtypes (m1–5).

Many types of neurotoxins, which block nerve transmission, have been isolated from the venoms of Elapidae (cobras, mambas, and kraits), Hydrophiidae, Crotalidae, and Viperidae. Among them, the presynaptic and postsynaptic types of neurotoxins are the most studied. The presynaptic type neurotoxins, which are often called β-toxins, ultimately inhibit the release of acetylcholine from nerve terminals by an unknown mechanism. All β-toxins have phospholipase A_2 activity, which is essential for the presynaptic neurotoxicity. Although more than one hundred phospholipases A_2 have been isolated from snake venoms, only a few show neurotoxic action. Unlike presynaptic toxins that possess phospholipase A_2 activity, all postsynaptic neurotoxins are nonenzymatic and bind irreversibly to nAChR, resulting in the blockage of the nerve transmission.

Although snake toxins, which associate with the nAChR, have been extensively studied and documented, very few studies on the interaction of snake toxins with the mAChR have been carried out. A few components which bind selectively to mAChR were recently isolated from the venom of the green mamba, *Dendroaspis angusticeps*. The toxins, designated as muscarinic toxins (MTXs), are small peptides consisting of 65 or 66 amino-acid residues. The three-dimensional solution structure is very similar to those of short-chain postsynaptic neurotoxins; the overall folding consists of three loops stabilized by the four disulfide bonds and forming a two- and a three-stranded β-sheet. Snake venoms that inhibit muscarinic AChR are actually quite common and are shown in *Table 1*.

Table 1. *The Abilities of Snake Venoms to Inhibit [^{3}H]QNB Binding*

Snake venom	[^{3}H]QNB-bound [fmol]	Inhibitory rate [%]
Elapidae		
Bungarus caeruleus	13	71.3
Bungarus fasciatus	9	83.0
Dendroaspis angusticeps	10	81.4
Hemachatus haemachatus	15	65.5
Naja naja	13	71.0
Naja naja philippinesis	8	87.8
Naja naja samarensis	12	74.5
Naja naja sputatrix	7	88.7
Ophiphagus hannah	29	15.9
Hydrophiidae		
Lapemis hardwickii	28	30.1
Laticauda semifasciata	25	38.8
Crotalidae		
Agkistrodon acutus	10	79.7
Bothrops atrox	9	83.2
Crotalus adamanteus	30	22.9
Crotalus atrox	7	88.4
Crotalus viridis viridis	10	82.3
Trimeresurus flavoviridis	10	79.7
Saline	38	0.0
Saline + µM Atropine	3	100.0

The active component was isolated from *N. naja sputatrix* venom [49].

Both the crude venom and purified muscarinic inhibitor suppressed QNB (quinuclidinyl benzilate)-receptor interaction in a dose-dependent manner. When the purified preparation was used, a concentration of muscarinic inhibitor as low as 0.1 µg/ml caused sigificant inhibition of QNB binding. *N. naja sputatrix* muscarinic inhibitor was found to have much more inhibitory potential than MTXs from *D. angusticeps* which require 5.0 µg/ml for the inhibitory action [50].

The molecular weight of purified muscarinic inhibitor was estimated to be 16 kDa. By SDS-PAGE, it was determined to be 16 kDa without β-mercaptoethanol and to be 14 kDa with the reducing agent. Since the molecular weight estimated under reduced condition was roughly identical with that under nonreduced condition, *N. naja sputatrix* muscarinic inhibitor is a monomeric single-chain protein. By mass-spectrometric determination, the molecular mass was confirmed to be 13633 Da.

The sequence for the first 16 residues was determined to be Asn-Leu-Tyr-Gln-Phe-Lys-Asn-Met-Ile-Gln-Cys-Thr-Val-Pro-Asn-Arg. Comparing the amino-acid sequence with many snake-venom proteins revealed that the primary structure of the muscarinic inhibitor was very similar to those of phospholipases A_2 from the genus *Naja*. The phospholipase A_2 assay indicated that *N. naja sputatrix* muscarinic inhibitor is an active phospholipase A_2.

3. Potassium-Channel-Binding Neurotoxins

The potassium channel plays an important role in the repolarization process in nerve transmission and is less well known than the sodium channnels in the nerve. The K^+ channel is composed of membrane proteins and has six transmembrane helical regions. Both the NH_2- and COOH-terminal chains are located inside the membrane. The first snake toxin found to bind K^+ is dendrotoxin. Dendrotoxin and other K^+-channel-inhibiting toxins are shown in *Table 2*. This toxin is a potent convulsant and facilitates transmitter release by inhibition of voltage-sensitive K^+ channels [51a–f].

Dendrotoxins are more suitable for study of the K^+ channels than β-btx, because they lack the intrinsic phospholipase A activity [52]. Dendrotoxin induces repetitive firing in rat-visceral sensory neurons by inhibiting a slowly inactivating outward K^+ current [53].

Dendrotoxin (DTX) has an M_r of 7000 [54] and strongly binds to synaptic plasma membranes of rat or chick brain [55]. The receptor has high M_r of 405000–465000 [56]. *Rhem* and *Lazdunski* [57] also isolated the K^+-channel proteins that bind to DTX I. By using neuraminidase and glycopeptidase, K^+-channel proteins that bind to DTX, β-btx, and MCD (most cell degranuting peptide) were reduced to 65000 Da. This indicates that a peptide core of the K^+-channel protein that binds to the toxins is *ca.* 65000 Da [58]. β-Bungarotoxin, normally considered to be a presynaptic neurotoxin affecting the nerve ending, is also a K^+-channel blocker [59a, b]. There are considerable sequence homologies between β-bungarotoxin and dendroaspis-venom toxins. The K^+-channel inhibitory action of β-btx is independent of its phospholipase A activity [60]. *Alvarez* and *Garcia-Sancho* [61], using crude venoms of *Notechis scutulatus*, *Oxyuranus scutulatus*, and *Vipera russelli*, found that they did inhibit K^+ channels. The first two venoms are known to contain potent presynaptic toxins. One should, however, notice that *Alvarez* and *Garcia-Sancho* used K^+ channels of red cells, whereas most other studies were done on synaptosomes. *Anderson* and *Harvey* [62] used other tissues, such as diaphragm and the nerve-muscle preparation, and observed the same inhibition as in synaptosomes studied by many other workers.

Table 2. *Potassium-Channel-Binding Neurotoxins*

Venom	Name	Identical with
Dendroaspis angusticeps	α-DaTX	Dendrotoxin, $C_{13}S_2C_3$
	β-DaTX	New toxin
	γ-DaTX	New toxin
	δ-DaTX	$C_{13}S_1C_3$
D. polylepis polylepis	DTX_14	Toxin I
Bungaris multicinctus	β-btx	β-Bungarotoxin

4. Antiacetylcholinesterase Neurotoxins

The fourth type of neurotoxin is the one that binds to acetylcholinesterase [63a, b]. When acetylcholinesterase is not functioning, acetylcholine (after binding to the acetylcholine receptor) cannot be hydrolyzed; consequently, normal nerve transmission is impaired. Acetylcholinesterase action of *D. Angusticeps* venom was first reported by *Rodriguez-Ithurralde et al.* [64].

Fasciculin forms a complex with acetylcholinesterase, and the toxin in the complex is very similar to the isolated toxin structure [65].

Antiacetylcholinesterase-type neurotoxins have so far only been isolated from African mambas (*Denndroaspis*). The names of the snake venoms from which anticholinesterase-type toxin was isolated are shown in *Table 3*.

Anticholinesterase-type neurotoxin has 57–60 amino acids in a single polypeptide chain, cross-linked by three disulfide bonds. Fasciculin 2 is identical with toxin F_7 isolated by *Viljoen* and *Botes* [66]. Similarly, toxins C and D from *D. polylepis polylepis* venom are also related to acetylcholinesterase-type neurotoxin [67a, b]. Although anticholinesterase neurotoxins are structurally similar to postsynaptic type neurotoxins and cardiotoxins, they differ immunologically.

The crystalline structure of fasciculin 2 indicates that the toxin is structurally related to both cardiotoxins and α-neurotoxins. Fasciculin 1 was also examined by X-ray crystallography [68].

The toxin binds to acetylcholinesterase and renders acetylcholine unhydrolyzed. This causes continuous excitement of the muscle. The inhibition of acetylcholinesterase is seen not only *in vitro*, but also *in vivo*. For instance, 80% of the acetylcholinesterase activity in the *locus coeruleus* was inhibited by the injection of fasciculin 2 in rats [69]. The inhibition of the enzyme by fasciculin is long-lasting, and a 74% inhibition five days after injection was observed [70]. By inhibiting acetylcholinesterase, fasciculin increased the amplitude and time course of the end-plate potential [71]. Fasciculin also increased the amplitude of the miniature end-plate potential [64b]. Acetylcholinesterase enveloped in an artificial liposome can also bind to fasciculin [72]. Because of the inhibition of acetylcholinesterase, dendrotoxins or other facilitatory toxins enhance the release of acetylcholine. Thus, dendrotoxins and fasciculins have synergistic action that enhances the lethality. Fasciculin 2 has no presynaptic action on transmitter release or on postsynaptic receptor-blocking action; the main action is on anticholinesterase [73]. There was no significant change in dopamine or serotonin concentration in rats after fasciculin 2 injection [74].

This work was supported by NIH MERIT Award 5R37GM15591.

Table 3. *Antiacetylcholinesterase-Type Neurotoxins*

Venom	Toxin
Dendroaspis angusticeps	F_7, Fasciculin
D. polylepis polylepis	C

Received: November 28, 1997

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